## 10/585299 NL0500026 AP20 Rec'd PCT/PTO 06 JUL 2006

## CLAIMS

- 1. A method for in vitro growing of connective tissue substitute, said connective tissue substitute being populated with fibroblasts, said connective tissue substitute being suitable for application onto a wound in a subject in need thereof, said method comprising the steps of:
  - a) providing a connective tissue layer substantially free of living cells having a first and a second contacting side, the first contacting side being opposed to the second contacting side;
  - b) placing the connective tissue layer in a container comprising fibroblasts, allowing the fibroblasts to contact the first contacting side of the connective tissue layer; and
  - c) at least temporarily simultaneous with step b) contacting the second contacting side of the connective tissue layer, the connective tissue layer being positioned in the container, with an intact epithelial layer, to attract said fibroblasts into the connective tissue layer by passing through the first contacting side of the said connective tissue layer.
- 2. A method according to claim 1, wherein the container comprising fibroblasts is a cell culture dish or a transwell comprising fibroblasts.
- 3. A method according to any of claims 1 or 2, wherein the second contacting side of the connective tissue layer is kept substantially free from contact with fibroblasts from the container.
- 4. A method according to any of the preceding claims, wherein the fibroblasts are a primary cell culture.

- 5. A method according to any of the preceding claims, wherein the intact epithelial layer is an intact epidermal layer.
- 6. A method according to any of the preceding claims, wherein the intact epithelial layer is derived from the said subject.
- 7. A method according to any of claims 1-4 or 6, wherein the epithelial layer is derived from the tongue, oesophagus; the oral cavity, the cornea of the eye, respiratory tract or intestinal cavity.
- 8. A method according to any of the preceding claims, wherein the intact epithelial layer is obtained from one or more skin biopsies of said subject.
- 9. A method according to any of claims 1-4 or 6-7, wherein the intact epithelial layer is obtained from one or more oral biopsies of said subject.
- 10.A method according to any of the preceding claims, wherein the second contacting side of the connective tissue layer comprises a basement membrane.
- 11.A method according to any of the preceding claims, wherein the fibroblasts are obtained from one or more skin biopsies of said subject.
- 12.A method according to any of the preceding claims, wherein the fibroblasts are obtained from one or more oral biopsies of said subject.
- 13.A method according to any of the preceding claims, wherein the fibroblasts and intact epithelial layer are derived from the said subject.

- 14.A method according to any of the preceding claims, further comprising the step of introducing one or more nucleotide sequences into the fibroblasts and/or intact epithelial layer.
- 15.A method according to any of the preceding claims, said connective tissue layer substantially free of living cells being derived from a donor organism, said subject not being said donor organism.
- 16.Connective tissue substitute obtainable by the method of any of claims 1-15.
- 17.A method for closing of a wound, comprising the step of applying a connective tissue substitute according to claim 16 onto a wound.
- 18.A method according to claim 17, wherein the wound is a chronic wound or an acute wound.
- 19.A method according to claim 18, wherein the chronic wound is chosen from the group, consisting of a venous ulcer, arterial ulcer, diabetic ulcer, decubitus and persisting burn wound.
- 20.A method according to claim 18, wherein the acute wound is chosen from the group, consisting of a surgical wound, accidental wound, decubitus and burn wound.
- 21.A method for treating a subject suffering from a wound, said method comprising applying the connective tissue substitute according to claim 16 onto said wound.